

SHORT NOTE ON THE MECHANISM OF HAEMOLYSIS IN *PIROPLASMOSIS* *CANIS*

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For the following researches puppies and dogs of various ages were infected with our laboratory strain of *Piroplasma canis*. The infection was invariably fatal for *young* puppies up to the age of 2½ months. After a varying incubation period, the animals shewed parasites in the blood in small numbers at first, but very soon a rapid multiplication occurred and lasted for 24 to 36 hours, accompanied by haemoglobinuria. The urine was of a light reddish to dark mahogany colour, and the more rapid the multiplication of the parasites, the darker was the colour of the urine. Pronounced jaundice of the tissues was only noticed in two out of seventy animals used. In these young animals the blood serum, taken at death, was of a dark reddish to mahogany colour, according to the colour of urine.

In dogs and older puppies, the disease was hardly ever fatal. The parasites did not multiply so rapidly, and nearly always were present in smaller numbers; we have never observed a pronounced haemoglobinuria in these animals, but for three weeks after inoculation the serum was slightly reddish in colour.

It was especially noticed that in young puppies a rapid diminution of the blood corpuscles took place during the last few hours before death.

It seemed, therefore, of interest to determine whether the destruction of the blood corpuscles was due to Isolysins or Autolysins. With this object, the serum of infected animals in different stages of the disease was added in varying dilutions to red blood corpuscles of

the infected animals from which the serum was obtained, and also to blood corpuscles of normal dogs or puppies.

The serum was used both fresh and after being heated for thirty minutes at a temperature of 56° C.; and in this latter case with and without the addition of complement (fresh centrifuged dog serum).

The blood corpuscles were usually washed three times in 0.9% saline solution, and used in a 10% suspension.

Serum of a dark reddish colour taken from a puppy three hours before death: haemoglobinuria marked.

	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
A. 10 per cent. suspension of infected dog's red blood corpuscles	1	1	1	1	1	1
Fresh normal dog's serum (diluted 1 in 10)	1	1	1	1	1	1
Infected dog's inactivated serum	1	0.5	0.1	0.05	0.01	0.005
Two hours at 37° C.	Slight Haemolysis	0	0	0	0
B. 10 per cent. suspension of infected dog's blood corpuscles	1	1	1	1	1	1
Infected dog's serum	1	0.5	0.1	0.05	0.01	0.005
Two hours at 37° C.	Slight Haemolysis	0	0	0	0
C. 10 per cent. suspension of infected dog's blood corpuscles	1	1	1	1	1	1
Normal serum	1	0.5	0.1	0.05	0.01	0.005
Two hours at 37° C.	0	0	0	0	0	0
D. 10 per cent. suspension of normal dog's blood corpuscles	1	1	1	1	1	1
Fresh normal dog's serum (diluted 1 in 10)	1	1	1	1	1	1
Inactivated infected dog's serum	1	0.5	0.1	0.05	0.01	0.005
Two hours at 37° C.	0	0	0	0	0	0

		c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
E. 10 per cent. suspension of normal dog's blood corpuscles	1	1	1	1	1	1
Infected dog's serum	...	1	0.5	0.1	0.05	0.01	0.005
Two hours at 37° C.	...	0	0	0	0	0	0

Similar experiments were carried out with the serum of a larger dog, withdrawn during the early acute stage of the infection, when parasites were present in small numbers. The serum was of a light reddish colour. The results were entirely negative: in none of the test tubes was there any sign of haemolysis.

The series of experiments was repeated in the same way as in the first experiment, with the serum of a puppy; the serum of which was of a dark brownish mahogany colour; and also in these experiments, 1 c.c. of the infected serum added to 1 c.c. of 10% infected blood corpuscles, with both activated and inactivated serum, caused an extremely slight haemolysis, whereas in no other tube was there any sign of haemolysis noticed.

The same experiment was repeated with the serum of a dog three weeks after the first appearance of the parasites, and also with the serum of a puppy at the commencement of the rise of temperature. In no case was there any haemolysis of either normal red blood corpuscles, or of the red corpuscles of the infected animal from which the serum was obtained.

CONCLUSION

These experiments tend to prove that the haemolysis and the haemoglobinuria in infections with *Piroplasma canis* is due neither to an Isolysin nor to an Autolysin, but apparently only to a disintegration of red blood corpuscles after the escape of the parasites from them.